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The Effects of Methyl Jasmonate on Calcium Excess: Changes in Mineral Compounds and Physical-Biochemical Parameters in American Grapevine Rootstocks

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Abstract The stress situation occurs when plants encounter conditions that are unsuitable for them. Abiotic factors are the most common stressors. In abiotic stresses, imbalances in the nutrient intake by plants have an important share. This situation, which is observed when the intake of some elements is restricted, or conversely, the solubility of some of them increases and becomes toxic, can cause yield and quality losses, and in extreme cases, the death of plants. It is common for high-lime soils to have high pH, thus, nutrient intake problems. This situation brings with it many negatives. Chlorosis is observed in this type of soil, the string intervals of young leaves are yellowing and root development becomes limited. Applications used in soil neutralization, such as leonardite, humic acid, and micronized sulfur, are applications that cannot give results in a short time. It is therefore of great importance that the plant endures in these environments. Today there is a focus on genetic engineering for this purpose. But these methods are expensive, requiring know-how. So finding natural, easy-to-use, practical, and harmless alternatives to human health is at the forefront. Plants are known to synthesize certain hormonal compounds that act as signaling molecules under stress, and therefore some growth regulators are used to provide stress resistance. These signals include jasmonic acids. Jasmonic acid, or its methyl ester, jasmonates, are compounds considered plant hormones, with a multifaceted effect being stimulating, inhibitory, and protective. This research also investigated the effects of exogenous methyl jasmonate applications in three American vine rootstocks (5 BB, 41 B, and 1103 P) that differ in lime sensitivities in lime-containing environments. Cuttings were planted in to polyethylene pots prepared by adding equal volumes of sterile pearlite and turf and placed in the greenhouse for the growing period. They are systematically irrigated with ½ MS solution. After two months following planting, rooting, and leafing of the cuttings, calcium oxide (0, 10, 25, and 40% CaO) was applied to root regions. MeJA (150, 300, and 450 ppm) in spray form, was applied to all leaves. A two-month period of improvement was followed after the MeJA application, the trial was terminated. Physical (shoot weight, shoot length, the average number of leaves per shoot, level of damage, rooting rate) and biochemical (chlorophyll amount, degree of membrane injurity, proline amount, total phenolic and mineral compound content) parameters were conducted to measure the level of stress in plants. Data obtained from the study reveal that MeJA has the potential to be used to counter stress caused by lime in American grapevine rootstocks.

Keywords Grapevine rootstock, Methyl jasmonate, Phenolic compounds, Mineral compounds

1. Introduction

The grapevine is one of the world's economically important fruit species. Because it is less selective than many cultural plants for climate and soil requirements and is one of the oldest agricultural crops of human beings, its cultivation is widely spread across the world. Turkey has a variation potential for vine genes along with the long-established culture of vineyard due to Turkey's location on the climate belt best suited for viticulture, at the intersection of the vine's gene centers [1]. Clay and lime content is known to be high in Turkey territory and organic matter content is generally low. Also, 14.14% of the territory of the Aegean region, 34.21% of the territory of the Mediterranean region, and 37.08% of the territory of the central-south region are very limy. As the amount of lime in the soil increases, deficiencies occur in the intake of other elements such as magnesium, mangan, and zinc, mainly iron. One of the most common nutrient deficiencies in viticulture is the lack of iron in soils with a lime content of more than 20% [2]. Hence, the iron deficiency is not only due to the inadequate amounts of iron

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in the soil, but also because it is not in useful form for plants. However, given that 26.87% of Turkey's territory is below 4.5 mg/kg, which is considered critical in terms of useful iron, iron deficiency in vineyards is inevitable. In case of deficiency, the string intervals of young leaves are yellowing, and the strings remain green [3]. Root development is also limited in these conditions, and this adversely affects the quality and yield of the plant. The vine plant is actually a plant that can be produced easily and successfully with cutting. But it is imperative to use American vine rootstocks for economic viticulture due to the Phylloxera pest. Hence, in the case of nutrient intake, the nutrition of the rootstock comes to the fore. The durability of the grapevine rootstocks varies between them but lags behind the varieties of the *V.vinifera* species.

Applications such as leonardite, humic acid, and micronized sulfur, which are used to neutralize limy soils, are applications that cannot give results in a short period of time, and it is of great importance to increase the resistance of the plant. It has been determined in recent years that the biotic and abiotic stress environments that plants encounter can be mitigated by offering biological approaches, or it can be given the plant durability in this way.

Plants are known to transmit a number of signals in their sensitivity processes to stressors. These signals include salicylic acid [4] ethylene [5], and jasmonates [6]. These signals also play a role in initiating defense mechanisms. It is therefore known that some growth regulators can be used to provide resistance to the stress environment [7,8].

Jasmonic acid (JA), which is a phytoalexin and forms the plant's active defense mechanism, is a compound [9] that is first isolated from the jasmine (*Jasminum grandifolium*) plant in the Oxylipids class. JA and its methyl ester, methyl jasmonate (MeJA) [10], are found in all higher plants [11]. Jasmonates are compounds that have multifaceted effects, including stimulating, inhibitory and protective effects, and are therefore considered by many scientists to be in the plant growth regulators class [12]. It is known in general that jasmonates promote root formation in plants [13], block enzymes that cause denaturation of proteins in injuries, are effective in signal molecules and increase β-Carotene synthesis [14], promote germination in seeds [15], are effective in secondary metabolism, affecting genes that regulate the formation of cell wall along with defensive proteins [16].

It is seen that the studies carried out on JA so far have focused on examining the effects on the yield and quality of plants, primarily on stress physiology. It has been stated that carbonic anhydrase activity related to the mechanism of defense against osmotic stress increases with JA [17], that JA improves photosynthetic performance in salt stress in barley [18], and that MeJA in strawberries increases the resistance against water stress [19]. MeJA was determined to delay flowering in long-day plants, inhibit proteinase enzyme activity, increase aromatic components and anthocyanins, and inhibit disease and harmful development [20]. Studies of JA or MeJA in the vine plant are usually intended to increase the quantities of secondary components or to determine the effect on certain diseases. Böttcher et al. [21] conducted a study to determine the effect of the plant hormone JA on grain development and maturation. Krisa et al. [22] stated that the addition of MeJA to Gamay and Cabernet Sauvignon cell cultures increased total piseids. Decendit et al. [23] found that the addition of MeJA to the medium of Gamay cell cultures led to increased flavanols and complex stilbene derivatives. It has been determined by studies that the synthesis of anthocyanin is increased with JA application in Gamay cell cultures [24], again, MeJA application in grapes promotes the accumulation of resveratrol [25], MeJA in the Negramaro variety is effective in increasing the amount of stilbenes [26]. Oçkun [27] indicated that JA and salicylic acid are effective in the formation of the callus in the vine. Malabarba et al. [28] studied the role of jasmonates in tendril movement in the vine and determined that even without mechanical stimulants, jasmonates showed strong activity in triggering tendril attachment. A study conducted in cell suspension cultures in vine also found that MeJA, administered at different doses, promotes tocopherol production [29].

Apart from this, there are studies to determine the effects of JA and MeJA applications against biotic stressors in the vine. One of these studies investigated the role of JA in gaining resistance to *P. viticola*. The research indicated that gene expressions associated with the JA and SA signaling pathways increased in the early hours after *P. viticola* vaccination, hence JA was effective in gaining the defense status against the biotrophic pathogen [30]. It is also known that the level of JA increases rapidly after infection in resistant cultivars [31,32], while this increase is limited in susceptible cultivars. Fang et al. [33] conducted a study on *Vitis amurensis* and stated that drought tolerance can be increased by regulating JA synthesis. In the study of Gülbasar Kandilli et al. [34], the vines were inoculated with Powdery mildew and Downey mildew, SA, JA, and abscisic acid. They were found to be in high quantities shortly after inoculation with pathogens, especially in resistant varieties. In short, JA or MeJA is prominent in studies related to stress.

This research is intended to determine how MeJA, a jasmonic acid ester in vine plants grown in lime-containing environments that creates stress in the plant, affects mineral intake, and thus produces many negative consequences. Three American grapevine rootstocks with different sensitivity to lime were selected and the effects of MeJA applications on rootstocks in environments containing different levels of lime were investigated.

2. Material and Methods

The research was carried out between 2019-2020 in Yozgat Bozok University, Faculty of Agriculture, Department of Horticulture.

2.1. Material

The research used Kober 5 BB (5 BB), 41 B M.G. (41 B), and 1103 Paulsen (1103 P) American vine rootstock materials, which were obtained from Bursa Agriculture Inc (Bursa/TURKEY).

5 BB: It is a hybrid of berlandieri x riparia. It is a strong rootstock and can fit moist and clay soil. It rests well on around 20% active lime, not liking very arid soils.

41 B: It is a vinifera x american hybrid. With a short vegetative period, the rootstock has an excess resistance to lime and is especially used for extremely chalky soils.

1103 P: It is berlandieri x rupestris hybrid. The rootstock, which develops vigorously and adapts well to clayey-lime soils, is resistant to active lime up to 17-18%.

2.2. Methods

The preparation of cultivation environment, planting, and applications of lime, and MeJA: The research was planned according to the randomized block design with 3 repetitions and there were 10 plants per repetition. Cultivation environments were prepared by adding equal volumes of sterile pearlite and turf to pots made primarily of polyethylene material. American vine rootstocks were planted in these environments and placed in the greenhouse for the growing period. They are systematically irrigated with ½ MS solution to meet water and nutrient needs in these environments. After two months following planting, rooting, and leafing of the cuttings, lime was applied to root regions, supplying 0, 10, 25, and 40% calcium oxide (CaO). Then the rootstocks were divided into groups, and the MeJA, crafted as 0 (control), 150, 300, and 450 ppm in spray form, was applied to all leaves. The control application was realized with water. A two-month period of improvement was followed after the MeJA application to rootstocks in environments with different lime contents, then the trial was terminated and measurements, observations, and analyses regarding physical and biochemical changes were made detailed below.

Physical Analyses

- *Shoot weight*: The shoot weight was measured with the help of analytical scales of 0.0001g precision in g.
- *Shoot length*: The shoot length was measured with the help of a ruler in cm.
- *Average number of leaves per shoot (ANLPS)*: All leaves on shoots were counted and determined.
- *Rooting rate*: The rooting rate was determined by the ratio of root-forming rootstocks to total rootstocks.
- *Level of damage*: The scale method created by Martinez Barroso and Alvarez [35] was modified and used. Plants that do not have chlorotic tissues resulting from the damage are "level 0"; light yellowing at the leaf edges are "level 1"; yellowings at more than 50% of the leaf are "level 2"; and chloroses which cause the death of the plant are described as 'level 3' damages.

Biochemical Analyses

- *Chlorophyll content:* Chlorophyll analyses are determined by Chlorophyll Meter in SPAD.
- *Degree of membrane injurity:* It was determined by measuring the excess electrolyte delivered to the outside from plant cells under stress conditions [36]. Membrane injurity index was calculated in percentage according to the formula below.

$$MII = (Lt\text{-}Lc/1\text{-}Lc) \times 100$$

Lt: EC value before autoclaving/EC value after autoclaving of treatment leaf

Lc: EC value before autoclaving/EC value after autoclaving of the control leaf

- *Proline content:* Determining the proline content in samples was made according to the method of Bates et al. [37]. Proline concentration was determined as μmol/g proline (fresh weight).
- *Total phenolic content analysis:* Extraction was performed according to the method of Kiselev et al. [38]. Total phenolic content analyses were based on Singleton and Rossi [39] using the Folin Ciocalteu colorimetric method. Spectrophotometer readings were performed at a wavelength of 765 nm. Total contents of phenolic compounds were given as mg/g (gallic acid equivalent (GAE)).
- *Determining the mineral compound content:*

In the research, phosphorus, potassium, calcium, magnesium, and iron were determined by the Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) device (Perkin Elmer Otima-8000). The plant samples were burned on the Milestone Start D device [40]. The two-stage temperature program for the burning process was performed. The operation conditions of the device are as follows; Rf power (W) 1450; Injector: Alumina 2 mm i.d.; Sample tubing: Standard 0.76 mm i.d; Drain tubing: Standard 1.14 mm i.d.; Quartz torch: Single slot; Sample capillary: PTFE 1 mm i.d.; Sample vials: Polypropylene; Source equilibrium delay: 15 sec; Plasma viewing: Axial; Processing mode: Peak area; Gases: Argon and Nitrogen; Shear Gas: Air. Wavelength of mineral compounds are as follows; phosphorus: 214,9; potassium: 766,4; calcium: 315,8; magnesium 279,0; iron: 238. Results were given as ppm.

2.3. Statistical Analyses

The data obtained as a result of the analyses in the research were tested using the SPSS 20.0 package software. The Duncan multiple comparison test was used to determine the differences between group averages, and the numerical values were interpreted accordingly.

3. Results and Discussion

The performances of the effects of MeJA application at different lime concentrations on three different American grapevine rootstocks are presented in the tables below on the basis of rootstock genotypes. The lowest weighted shoots of 5 BB in assessing shoot weights appear to be from plants grown in the highest lime-containing pots without the application of MeJA (Table 1). Here, the impact shown by lime without the MeJA stands out. No statistical difference in terms of shoot lengths of these plants was identified. Similarly, no statistical differences were detected in terms of the ANLPS. However, although any difference was not detected, there are numerical fluctuations between the treatment groups and it is thought that the differences will also be seen statistically if the administered scales of lime and MeJA doses are wider.

Table 1. Effects of MeJA on some physical and biochemical properties in 5 BB rootstock

MeJA (ppm)	*CaO (%)*	*Shoot weight (g)*	*Shoot length (cm)*	*ANLPS (piece)*	*Rooting rate (%)*	*Level of damage (scale)*	*Chlorophyll (SPAD)*	*MII (%)*	*Total phenolic compounds (mg/g)*	*Proline (µmol/g)*
0	0	6,91 a*	32,31**	6,67	79,20 b	0,00 b	23,78 a-d	13,71 h	5,99 bc	0,20 a-c
	10	6,53 ab	31,79	5,94	59,40 e	0,33 b	23,30 a-d	13,85 h	6,46 bc	0,18 a-d
	25	5,67 ab	31,18	5,57	48,58 f	1,00ab	20,91 b-d	42,51 b	5,01 c	0,18 a-d
	40	4,11 b	27,78	5,44	47,60 f	2,00 a	19,63 d	46,02 a	3,17 d	0,07 d
150	0	6,83 a	30,71	6,72	89,10 a	0,33 b	23,20 a-d	13,97 h	4,91 c	0,21 a
	10	6,50 ab	30,67	6,17	59,40 e	0,00 b	24,30 a-c	14,65 h	6,47 bc	0,14 cd
	25	5,89 ab	30,47	5,94	79,20 b	0,33 b	21,87 a-d	35,49 d	6,11 bc	0,21 ab
	40	5,48 ab	29,22	5,89	89,10 a	0,33 b	21,25 b-d	37,56 c	5,80 bc	0,16 a-d
300	0	5,95 ab	26,19	6,50	69,30 cd	0,00 b	24,62 ab	13,51 h	5,47 bc	0,16 a-d
	10	5,56 ab	25,40	6,11	79,20 b	0,66 b	21,57 a-d	14,80 h	5,42 bc	0,14 cd
	25	5,35 ab	25,43	5,42	61,67 de	0,66 b	20,87 b-d	24,56 g	7,00 b	0,17 a-d
	40	5,55 ab	25,16	5,67	59,40 e	0,66 b	20,63 b-d	26,00fg	5,04 c	0,17 a-d
450	0	5,88 ab	30,59	6,05	76,67 bc	0,00 b	25,53 a	14,54 h	6,93 b	0,15 b-d
	10	6,08 ab	30,08	5,94	75,50 bc	0,33 b	23,52 a-d	14,70 h	6,53 bc	0,17 a-d
	25	5,70 ab	30,25	5,83	59,00 e	0,66 b	21,20 b-d	27,24 f	10,45 a	0,16 a-d
	40	5,50 ab	29,11	5,50	58,83 e	1,00ab	20,25 cd	29,56 e	5,78 bc	0,18 a-d

ANLPS: Average number of leaves per shoot, MII: Membrane injury index. * There is a difference between the averages expressed in different letters in the same column at a 5% significance level (p≤0.05), ** The difference between applications is not significant.

Table 2. Effects of MeJA on some physical and biochemical properties in 41 B rootstock

MeJA (ppm)	*CaO (%)*	*Shoot weight (g)*	*Shoot length (cm)*	*ANLPS (piece)*	*Rooting rate (%)*	*Level of damage (scale)*	*Chlorophyll (SPAD)*	*MII (%)*	*Total phenolic compounds (mg/g)*	*Proline (µmol/g)*
0	0	6,80 bc*	29,63 c	8,58 **	79,20 c-e	0,00	24,22 a-c*	11,57 fg	4,80 bc	0,13 e
	10	6,51 bc	29,63 c	8,75	71,87 ef	0,33	23,18 a-d	11,47 g	3,47 c	0,14 de
	25	6,42 bc	28,42 c	8,50	70,53 ef	0,66	23,08 a-d	13,24 c-e	4,34 bc	0,14 de
	40	5,88 bc	28,77 c	8,33	59,40 f	0,66	20,27 d	14,45 c	4,44 bc	0,18 b-e
150	0	6,62 bc	31,25 bc	9,17	99,00 a	0,00	23,93 a-c	9,65 h	5,50 b	0,17 b-e
	10	6,44 bc	30,28 bc	8,67	72,63 e	0,33	23,62 a-c	15,43 b	5,19 bc	0,16 c-e
	25	5,94 bc	30,75 bc	9,00	69,30 ef	0,33	23,53 a-c	15,83 b	5,10 bc	0,17 b-e
	40	5,29bc	30,57 bc	9,00	66,67 ef	0,66	21,47 cd	17,49 a	6,01 b	0,22 ab
300	0	5,87 bc	33,17 bc	8,00	99,00 a	0,33	25,43 a	10,34 h	4,65 bc	0,17 b-e
	10	5,39 bc	34,62 a-c	9,17	89,10 a-d	0,00	22,58 a-d	12,24 e-g	5,72 b	0,18 b-e
	25	4,90 c	34,73 a-c	9,00	85,67 b-d	0,33	22,63 a-d	15,83 b	5,58 b	0,17 b-e
	40	4,34 c	34,03 a-c	8,67	78,00 de	0,66	21,90 cd	12,52 d-f	5,86 b	0,25 a
450	0	9,51 a	42,50 ab	9,33	96,67 ab	0,33	25,20 ab	11,44 g	5,47 b	0,18 b-e
	10	7,53 ab	45,17 a	9,00	92,43 ab	0,33	22,18 b-d	11,97 fg	8,55 a	0,19 a-d
	25	6,86 bc	38,95 a-c	9,17	91,67 a-c	0,33	21,58 cd	13,33 c-e	5,96 b	0,21 a-c
	40	5,58 bc	31,00 bc	9,33	89,10 a-d	0,66	21,12 cd	13,70 cd	7,73 a	0,25 a

ANLPS: Average number of leaves per shoot, MII: Membrane injury index. * There is a difference between the averages expressed in different letters in the same column at a 5% significance level (p≤0.05),** The difference between applications is not significant.

Table 3. Effects of MeJA on some physical and biochemical properties in 1103 P rootstock

MeJA (ppm)	*CaO (%)*	*Shoot weight (g)*	*Shoot length (cm)*	*ANLPS (piece)*	*Rooting rate (%)*	*Level of damage (scale)*	*Chlorophyll (SPAD)*	*MII (%)*	*Total phenolic compounds (mg/g)*	*Proline (µmol/g)*
0	0	6,12 ab*	30,02 a	7,33 a	69,30 cd	0,33 de	24,95 a	12,88 j	3,78 gh	0,16 a
	10	5,45 ab	29,25 a	6,00 a	59,17 d	1,66 a-d	22,12 b-e	23,20 g	3,70 g-i	0,11 a-d
	25	4,63 ab	15,08 d	5,67 ab	59,30 d	2,33 ab	19,30 ef	39,62 a	2,91 hi	0,05 cd
	40	4,14 b	14,33 d	4,17 b	39,60 e	2,66 a	17,37 f	40,12 a	2,72 i	0,05 d
150	0	6,38 ab	28,90 a	7,00 a	62,73 d	0,33 de	24,28 a-c	14,24 i	4,41 fg	0,17 a
	10	5,59 ab	29,47 a	6,33 a	63,33 d	1,00 b-e	21,58 de	21,90 g	7,02 b	0,10 a-d
	25	5,18 ab	18,22 bcd	6,17 a	63,67 d	2,00 a-c	19,90 ef	38,67 ab	4,85 ef	0,14 a
	40	4,99 ab	16,25 cd	5,50 ab	61,30 d	2,00 a-c	19,77 ef	38,07 bc	4,60 e-g	0,13 ab
300	0	5,69 ab	25,37 ab	7,17 a	90,67 ab	0,33 de	24,72 ab	14,80 i	5,12 d-f	0,16 a
	10	5,52 ab	24,33 ab	6,00 a	85,87 a-c	0,66 c-e	21,93 c-e	22,93 g	3,09 hi	0,14 a
	25	5,45 ab	24,97 ab	6,00 a	76,67 b-d	1,66 a-d	20,73 e	36,00 de	5,54 c-e	0,10 a-d
	40	5,17 ab	24,33 ab	6,17 a	71,83 b-d	1,33 a-e	20,80 e	36,67 cd	5,97 cd	0,10 a-d
450	0	6,46 a	26,52 a	7,00 a	99,00 a	0,00 e	24,02 a-d	13,12 ij	6,30 bc	0,15 a
	10	6,03 ab	26,13 a	6,33 a	87,27 a-c	0,66 c-e	21,23 e	19,67 h	8,13 a	0,13 a-c
	25	5,71 ab	24,17 ab	6,17 a	87,27 a-c	1,33 a-e	20,60 e	32,69 f	6,18 bc	0,09 a-d
	40	5,43ab	22,67 a-c	6,50 a	83,93 a-c	1,00 b-e	21,57 de	34,91 e	5,08 d-f	0,06 b-d

ANLPS: Average number of leaves per shoot, MII: Membrane injury index. * There is a difference between the averages expressed in different letters in the same column at a 5% significance level (p≤0.05).

Another criterion is the rooting rate, and this criterion, determined in percentage, appears to be the highest in plants without lime and with the highest lime in the 150 ppm MeJA-treated group. Rooting was very low in plants that were not treated with MeJA but treated with 25% and 40% lime, revealing the role of MeJA in the effect of high lime on rooting. In the level of damage, which is visually detected, where the overall appearance of plants is assessed and a value is given as a scale, it is noteworthy that higher values were observed in the groups that did not receive MeJA and in which 25% and 40% lime was applied. The fact that damage is high in plants in environments with high two doses of lime and without MeJA suggests that MeJA is effective in preventing damage. The highest dose of both lime and MeJA also appears to produce similarly high levels of damage. Given the increasing doses of MeJA and the increasing damage in high lime environments, it is thought that it will be effective to conduct different dose trials in smaller ranges.

The research included analyses on the determination of chlorophyll, degree of membrane injurity, total phenolic compound, and proline content, which are from biochemical analyses that enable the acquisition of clearer data in measuring stress responses in plants compared to physical properties. Chlorophyll contents at high doses of lime in 5BB rootstock appear to remain at low levels. Membrane damage detected by the amount of electrolytes delivered outward from cell membranes is expected to be high in plants in the high-lime-containing environment. The low value in all groups administered 40% MeJA is an indication of the effect of MeJA in mitigating damage. The phenolic content, which is an important indicator in the measurement of stress in plants, was at the highest level in the 25% level of lime in the highest dose group of MeJA, which shows that MeJA is effective in tolerating stress.

Proline, an amino acid synthesized as an osmotic regulator in plants, is of great importance in protecting from the stress environment. The increase in proline indicates that stress exists in the environment and that the plant tolerates it. Therefore, the fact that this value appeared to be very low in a high-dose lime environment with no MeJA administration in 5BB rootstock suggests that the response to stress is low. It is also noted that there is no distinct difference between the implementation groups. While varying by rootstock genotype, it is thought that this difference may also be more pronounced by keeping the identified dose diversity wider.

In Table 2, where 41 B rootstock features are presented, it appears that in environments with the highest dose of MeJA, 0% and 10% lime, shoots develop better than any other application and attain a high weight. It is also noted that shoots in the same treatment are also involved in the longest shoots. Apart from that, in the 300 ppm MeJA treatment, plants appear to grow longer in all lime doses.

In terms of the ANLPS and the level of damage no statistical difference between the averages has emerged. Rooting rates remain at low levels regardless of lime doses in all plants without MeJA treatment. Biochemical changes of 41 B rootstock, which is best genotype adapted to limy environments, is examined, it is seen that the chlorophyll content remains in low levels at high lime doses. In addition, although not statistically, the numerically lowest chlorophyll value was seen in the plants with the highest lime dose without MeJA, which shows the effect of MeJA in this genotype as well. It is notable that there is less electrolyte leaking from membranes in plants without lime treatment but with 150 and 300 ppm MeJA treatment. Even if there is no stress caused by lime in the environment, the effect of MeJA, a plant hormone, in this way is seen. Greater synthesize of phenolic compounds in plants at 450 ppm MeJA and 10% and 40% lime doses indicates this tolerance. As another tolerance criterion, proline was synthesized at the highest lime contents at 150 and 300 ppm MeJA doses and at 450 ppm MeJA in all lime environments, which indicates the adaptation of the plant to stress.

The resistance of 1103 P to lime is relatively lower than that of the other two rootstocks. The effect of MeJA at different lime doses is evaluated. The shoot weights are the lowest in the environment without MeJA but with the highest dose of lime (Table 3).

The shoots remained shorter in the 25% and 40% lime environments in the groups that were not treated with MeJA and in which low dose (150 ppm) MeJA was applied. While there is no statistical difference in terms of ANLPS, they numerically appear to be higher in all groups that are not treated with lime. It appears that the rooting rate of 1103 P cuttings remains at very low levels regardless of lime dose in plants that are not treated with MeJA and treated with MeJA of 150 ppm, the lowest dose. On the other hand, it is noteworthy that in all plants treated with the highest dose of MeJA and 300 ppm MeJA at low lime levels, the rooting rate was very high. The level of damage appears to be lowest in all lime-free environments. There are studies examining the effects of MeJA or jasmonic acid on the physical development of plants in the face of different stressors. A study examined the effect of MeJA on germination and seedling development in salinity stress in the basil plant [41]. The salinity of 100 and 200 mM caused a reduction in the shoot length, while methyl jasmonate application of 0.1 µM doses in the salt environment at 100 mM dose significantly improved the reduction in shoot extension. In addition, 100 and 200 mM salinity caused a decrease in shoot fresh weight, while MeJA application caused an increase in fresh weight. A study also examined the effect of the application of MeJA on leaves in tomatoes on physical and biochemical properties under salt stress. Salt-tolerant Rio Grande and salt-sensitive Savera varieties were grown in salty environments, followed by different doses of MeJA treatment (0.0, 10, 20, 30, 40, 50, 60 µM). MeJA in tomato plants in salty conditions increased physiological and biochemical resistance [42].

It is notable that chlorophyll of 1103 P, whose resistance to lime is lower compared to the other two genotypes, is high in all environments where there is no lime. Damages at high lime doses lacking MeJA were also high as expected, and damage was also observed at low MeJA dose and 25% lime dose. Phenolic contents, which are a benchmark of high levels of stress but also of defense, were also synthesized at the highest dose of MeJA in this genotype at higher levels. Another tolerance benchmark, proline, is also low in high lime doses lacking MeJA, to some extent indicating damage from lime.

MeJA is known to eliminate the negative effects of salinity stress on chlorophyll content as a stressor [43,44]. A study also investigated the effect of the application of MeJA over leaves under salt stress on the physical and biochemical properties of tomatoes. Salt-tolerant Rio Grande and salt-sensitive Savera varieties were grown in salty environments, followed by different doses of MeJA treatment. While chlorophyll a severely decreased in both genotypes under salt stress, the application of 60 µM MeJA on leaf increased the content of chlorophyll A by 1.37 times compared to those without MeJA treatment [42].

There have been several studies aimed at determining the effects of MeJA applications on secondary metabolism in plants. One of these studies investigated the effects of MeJA on fruit peel color parameters, anthocyanin content, ethylene biosynthesis, phenolic content, and antioxidant capacity in Braeburn apples. Trees were administered 1120, 2240, or 4480 mg/L MeJA at intervals of 1 and 2 weeks, 105 and 175 days after full bloom. The research eventually found that anthocyanin content, intrinsic ethylene concentration, phenolic content, and antioxidant capacity increased with MeJA concentrations [45]. Another study researched the effects of exogenous MeJA applications on secondary metabolite synthesis in *Centella asiatica*, *Galphimia glauca* and *Ruscus aculeatus*. The study investigated the impact of MeJA in the production of 2,3-oxidosqualene. The development rate of in vitro plants and their free sterol content were evaluated after 100 µM of MeJA application. The research eventually found that *G. glauca* had 2,3-oxidosqualene production 152 times higher than *C. asiatica*, and that MeJA could be used to promote enzymes associated with triterpene synthesis [46].

The influence of MeJA applications on total phenolic and flavonoids has been researched with many studies [47,48,49]. Along with MeJA applications, it was determined that secondary metabolites and thus antioxidant activity were significantly promoted in raspberry [50], loquat [51], pomegranate [52], plum [53], medlar [54] and Ortega (*Lepechinia caulescens*) [55]. Saracoglu et al. [56] and Rehman et al. [57] found a lower rate of total phenolic, flavonoids, and antioxidant activity in cherries and orange, respectively, treated with MeJA compared to the control. Boonyaritthongchai and Supapvanich [58] also found the effect of MeJA in pineapple to be insignificant.

MeJA applications also have implications for proline contents, an important parameter in measuring the stress states of plants. There have been many studies examining the impact of MeJA applications on proline content under salt stress. In these studies, MeJA applications were found to significantly increase the amount of proline in both salty and salt-free settings [59,60]. A study also investigated the effect of administering MeJA on the leaves of tomatoes under salt stress. Salt-tolerant Rio Grande and salt-sensitive Savera varieties were grown in salty environments, followed by different doses of MeJA treatments. Proline content was determined 2.12 times higher in salt stress compared to the plants not under salt stress following the MeJA application. Proline content was detected 1.68 times higher in 50 µM MeJA applications in plants in salt-free environments. The salt-resistant genotype "Rio Grande" contained 1.18 times more proline than the salt-sensitive Savera genotype [42]. Anjum et al. [61] also noted increased proline content in pepper under salt stress.

The growth of the Cd-hyperaccumulator *Solanum nigrum* L. against Cd stress and exogenous MeJA and their physiological response in the short term (7 days) were studied. The application of different concentrations of Cd indicated no stimulant effects in proline deposition in the leaves. Only the highest Cd application increased proline content at the roots. At 40 mg/dm^3 Cd concentration, 0.01 μM MeJA application severely increased leaf proline content compared to control. A similar effect was observed in the roots but the actual increase in the proline content was observed in the application of 0.1 μM MeJA [62].

The biggest problem with plants grown in soils containing high lime, hence high pH, is imbalances in nutrient element intake. There are also deficiencies in the intake of certain nutrient elements, especially iron. The research, therefore, studied the contents of P, K, Ca, Mg, and Fe that the leaves contained. Data on the effects of MeJA on leaf nutrient intake in lime-containing environments are presented below on the basis of genotypes.

Table 4. Effects of MeJA on mineral compounds (ppm) in 5 BB rootstock

MeJA (ppm)	***CaO (%)***	***P***	***K***	***Ca***	***Mg***	***Fe***
0	0	724,79 c*	2705,60 g	3988,63 e	1306,94 b	274,92 a
	10	634,80 e	3099,96 ef	3379,72 gh	1263,23 b	260,63 ab
	25	575,80 fg	2698,65 g	3587,32 fg	1155,95 cd	259,23 ab
	40	525,83 h	2216,88 h	4784,29 c	144,26 g	209,27 c
150	0	701,15 cd	3258,89 de	3773,08 ef	1267,21 b	281,78 a
	10	589,50 f	3328,42 d	4426,69 d	1617,85 a	265,59 ab
	25	99,99 l	4045,61 b	4547,88 cd	1341,71 b	279,09 a
	40	545,60 gh	4322,75 a	5334,60 b	1179,79 c	276,87 a
300	0	890,28 a	3246,97 de	978,93 k	1092,28 d	278,21 a
	10	551,56 f-h	2928,11 f	929,52 k	989,96 e	275,69 a
	25	251,07 j	864,06 i	3986,64 e	317,38 f	268,58 ab
	40	238,40 j	820,45 i	4030,35 e	301,34 f	268,66 ab
450	0	823,24 b	4109,18 b	3052,91 i	1549,31 a	255,42 ab
	10	422,72 i	2187,08 h	3141,32 hi	1002,28 e	252,60 ab
	25	667,48 de	794,53 i	1312,19 j	345,89 f	242,08 b
	40	177,24 k	3735,69 c	5663,39 a	1012,32 e	261,39 ab

* There is a difference between the averages expressed in different letters in the same column at a 5% significance level ($p \leq 0.05$).

Table 5. Effects of MeJA on mineral compounds (ppm) in 41 B rootstock

MeJA (ppm)	***CaO (%)***	***P***	***K***	***Ca***	***Mg***	***Fe***
0	0	560,40 fg*	4875,04 b	2960,53 fg	1282,11 c	219,74 a-c
	10	504,67 g	2731,43 g	3230,72 e	1092,68 de	211,66 a-c
	25	106,43 k	337,20 hi	600,17 k	196,32 k	202,25 bc
	40	581,36 f	3748,60c	4173,39 c	929,27 gh	185,57 c
150	0	877,97 b	5119,40 a	3012,18 e-g	1625,80 a	223,13 a-c
	10	776,75 c	3616,49 cd	4028,36 c	1096,25 de	216,31 a-c
	25	635,59 e	3318,49 e	2995,30 e-g	1026,82 ef	222,00 a-c
	40	498,71 g	3025,46 f	5363,40 b	897,88 h	215,64 a-c
300	0	439,61 h	2870,50 fg	2789,68 g	1451,97 b	229,32 a-c
	10	530,80 g	3302,60 e	2991,32 e-g	1126,55 d	233,78 a-c
	25	333,72 ij	2638,06 g	2276,12 h	984,40 fg	244,98 ab
	40	528,61 g	3850,92 c	3104,56 ef	1330,78 c	256,84 a
450	0	355,67 i	1547,38 h	2019,84 i	524,69 j	201,57 bc
	10	1068,39 a	5282,31 a	3701,56 d	1168,87 d	207,61 bc
	25	300,99 j	1562,28 h	1711,91 j	516,84 j	209,46 a-c
	40	709,00 d	3427,76 de	5880,93 a	825,77 i	208,24 a-c

* There is a difference between the averages expressed in different letters in the same column at a 5% significance level ($p \leq 0.05$)

Table 6. Effects of MeJA on mineral compounds (ppm) in 1103 P rootstock

MeJA (ppm)	*CaO (%)*	*P*	*K*	*Ca*	*Mg*	*Fe*
0	0	550,07 d-f*	2805,93 h	3073,77 fg	1181,78 de	209,97 bc
	10	541,23 ef	3564,84 cd	2735,04 ij	1109,66 e-g	205,69 a-c
	25	588,81b-d	2995,65 gh	3462,16 e	1171,85 de	212,613 bc
	40	499,01 gh	3511,20 c-e	3018,14 gh	1144,03 d-f	208,55 c
150	0	573,81 c-e	4445,92 a	4803,16 b	1845,33 a	201,84 c
	10	673,34 a	2893,34 gh	4444,57 c	1504,61 b	200,21 c
	25	533,18 fg	3678,08 bc	3089,66 fg	1018,97 hi	210,35 bc
	40	695,29 a	3336,37 d-f	5159,77 a	1368,53 c	202,41 c
300	0	524,74 fg	3106,91 fg	2829,41 hi	1039,43 gh	228,70 a-c
	10	406,13 i	1935,77 j	2547,30 j	909,61 j	236,03 a-c
	25	619,11 b	3280,74 ef	3281,38 ef	1065,06 f-h	223,52 a-c
	40	590,60 bc	3840,98 b	3916,12 d	1221,51 d	215,95 bc
450	0	337,59 j	1733,13 j	2065,54 l	673,79 k	262,91 a
	10	481,63 h	4497,67 a	1740,22 m	990,07 h-j	244,983 ab
	25	468,12 h	2498,99 i	2306,92 k	951,62 ij	260,00 a
	40	591,79 bc	2829,77 h	3407,53 e	416,11 l	228,88 a-c

* There is a difference between the averages expressed in different letters in the same column at a 5% significance level ($p \leq 0.05$).

The highest P content was detected in 5 BB that were not treated with lime but 300 ppm MeJA (Table 4). Notably, P is found at lower levels in plants that were not treated with MeJA. It is also noted that P levels decrease as the amount of lime increases in the MeJA-free group. The highest content of K was reached in an environment containing 40% lime and in plants in the group where 150 ppm MeJA was applied. It was determined that the value here was almost twice as high as the plants that were not treated with MeJA in the same amount of lime environment. It is seen that the highest Ca is obtained from the environments where the highest lime and the highest MeJA application are performed. Mg was detected at the highest levels in plants in the environment where 10% lime existed and 150 ppm MeJA was applied and in plants that did not have lime but treated with 450 ppm MeJA. The amount of Fe in the leaves is noted that the values are statistically in the same group mostly. However, the fact that the highest content numerically occurred in the plants without lime but with 150 ppm MeJA treatment and the lowest Fe was found in the leaves of plants in the environment containing the highest dose of lime without MeJA, indicated the effect of MeJA on lime.

Plants with the highest intake of P in the 41 B genotype appear to be 10% lime-containing and 450 ppm MeJA-administered plants. Plants in the same group were also identified with the highest K intake. The highest K content was also statistically detected in plants that were not treated with lime but 150 ppm MeJA, while the plants in this group were also determined to be the plants in which the highest Mg was detected. 41 B were compared in terms of Fe intake on the basis of groups, it was observed that the Fe uptake was very low in plants without MeJA and in a high lime environment similar to 5 BB and that the lowest amount of Fe was found in the plants with the highest lime dose and without MeJA (Table 5).

In the 1103 P genotype, the highest P and Ca content was found in plants with 40% lime-containing groups and 150 ppm MeJA treatments; K appears to be highest at 150 and 450 ppm MeJA doses and in lime-free and 10% lime-containing environments respectively. It is notable that Mg occurs at the highest levels in the lime-free and 150 ppm dose of MeJA. Fe intake was observed at all lime levels, especially at the highest MeJA dose. The fact that high levels of Fe uptake were observed in the plants in the group not treated with MeJA at 10% lime level and at all lime doses except the highest lime at a dose of 300 ppm MeJA clearly shows the effect of MeJA on inhibited Fe intake (Table 6). However, no research was found in the literature that shows the effect of MeJA applications on mineral compound intake.

4. Conclusions

The high lime content is a condition seen in most of the land used in agriculture. Although vine is thought of as a plant that can more easily adapt to limy soils compared to many cultural plants, the negatives from lime are reflected in yields and quality. The most commonly used method for reducing the lime content of soils is the application of micronized sulfur, which is impractical if it is to be repeated and show its effect later, especially when it needs to be applied to vast lands. Therefore, approaches to increase the resistance of the plant to the stress environment are important. It is known that the application of jasmonate, a natural plant hormone, as a regulator of growth increases plant tolerance in a wide range of stress environments. Here, the effects of MeJA, a jasmonate, on the physical and biochemical properties of vine rootstocks, which have different resistance to lime, as well as their capacity for mineral uptake, were investigated, and the resulting data indicated the potential for use of MeJA in countering this stress. It is also thought that it would be effective to try different doses of MeJA, especially in different culture plants whose sensitivity to lime is known.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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